#41308
10/13/01

MVIEWM.005A

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Sherman, et al.	Group Art Unit 1652
Appl. No.	:	09/501,730	
Filed	:	February 10, 2000	
For	:	**AGGREGATE-FREE URATE OXIDASE FOR PREPARATION OF NON-IMMUNOGENIC POLYMER CONJUGATES**	
Examiner	:	Pak, Y.	

DECLARATION OF L. DAVID WILLIAMS, Ph.D.
SUBMITTED UNDER 37 C.F.R. § 1.131

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

1. I, L. David Williams, Ph.D., am a named inventor on the above-captioned patent application.
2. I have read and understood the specification and the claims of the above-captioned patent application.
3. I have read and understood Caliceti et al. *Bioconjugate Chem.* **1999**, *10*, 638-646, which has a web publication date of June 2, 1999.
4. Exhibit A, attached hereto, is a copy of several pages of one of my laboratory notebooks.
5. Pages 1 and 2 of Exhibit A show the results of a purification procedure that I conducted prior to June 2, 1999. The tracings shown on pages 1 and 2 are ion-exchange and size-exclusion chromatograms, respectively, of the product of my purification steps. The areas

under the peaks of the chromatograms on pages 1 and 2 show the relative abundance of several aggregates of uricase.

6. The areas under the peaks assigned to tetramers are much larger than the area under the peaks assigned to octamers or other aggregates. These data demonstrate the purification of uricase that is substantially free of aggregates larger than tetramers.

7. Therefore, prior to June 2, 1999 I had invented and was in possession the subject matter claimed in the above-captioned patent application.

8. I declare that all statements made herein are true, and that all statements made upon information and belief are believed to be true, and further, that these statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. § 1001, and that willful, false statements may jeopardize the validity of the application, or any patent issuing thereon.

October 4, 2001
Date

L. David Williams
L. David Williams, Ph.D.

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100301

19-52

linear5_M1_UV_280nm_01 — linear5_ConcB_01 —— Fractions_1

Edit - Run description

Sample DTG-Ply-KS-dN, 98%, 0.2u filtered
Loop load thru pump
Column MonoQ HR10/10
Eluent A 0.1M pH 10.3 $NaCO_3$
Eluent B 0.6M NaCl in Buffer A
Gradient Linear 0-100% C from 130 to 250 mL
Flow rate 1 mL/min
Fraction size 4 mL

METHOD

Method base : ml

***** Main method *****

0.00	CONC_B	0.0	()
0.00	FLOW	1.0	()
0.00	RECORDER_SPEED	0.50	()
130.00	CONC_B	0.0	()
250.00	CONC_B	100.0	()
270.00	CONC_B	100.0	()

RUN_LOG

Method file name LINEAR5.FMT
Run by LDW [redacted], 8:52:26 am
Config: Method base is ml
Config: Injection valve is valve No. 1
Config: Pump A&B is P-500, calibration 110.00
Config: Pump C is P-1, calibration 150.00
Config: UV-M -> Monitor 1, Input range = 100 mV

0.00		Method start. Result name LINEAR5.FRS	
0.00	Auto:	Tube No 1	
80.50	Auto:	Pause	10:13:02 am
80.50	Auto:	Continue	10:16:54 am
130.02	Auto:	Tube No 2	
134.02	Auto:	Tube No 3	
138.01	Auto:	Tube No 4	
142.02	Auto:	Tube No 5	
146.01	Auto:	Tube No 6	
150.01	Auto:	Tube No 7	
154.01	Auto:	Tube No 8	
157.98	Auto:	Tube No 9	
161.99	Auto:	Tube No 10	
165.99	Auto:	Tube No 11	
169.97	Auto:	Tube No 12	
170.05	Manual:	Hold	11:46:34 am
173.96	Auto:	Tube No 13	
177.96	Auto:	Tube No 14	
181.95	Auto:	Tube No 15	
182.03	Manual:	Continue	11:58:33 am
185.96	Auto:	Tube No 16	
189.96	Auto:	Tube No 17	
191.97	Manual:	Hold	12:08:30 pm
193.95	Auto:	Tube No 18	
197.94	Auto:	Tube No 19	
201.94	Auto:	Tube No 20	
205.94	Auto:	Tube No 21	
208.00	Manual:	Continue	12:24:32 pm
209.94	Auto:	Tube No 22	
213.93	Auto:	Tube No 23	
217.92	Auto:	Tube No 24	
221.93	Auto:	Tube No 25	
225.93	Auto:	Tube No 26	
229.92	Auto:	Tube No 27	
233.91	Auto:	Tube No 28	
237.91	Auto:	Tube No 29	
241.90	Auto:	Tube No 30	
245.90	Auto:	Tube No 31	
249.89	Auto:	Tube No 32	
253.89	Auto:	Tube No 33	
257.87	Auto:	Tube No 34	
261.86	Auto:	Tube No 35	
265.87	Auto:	Tube No 36	
269.87	Auto:	Tube No 37	
273.85	Auto:	Tube No 38	
277.85	Auto:	Tube No 39	
281.84	Auto:	Tube No 40	
285.84	Auto:	Tube No 41	
298.02	Auto:	Method end	1:54:38 pm

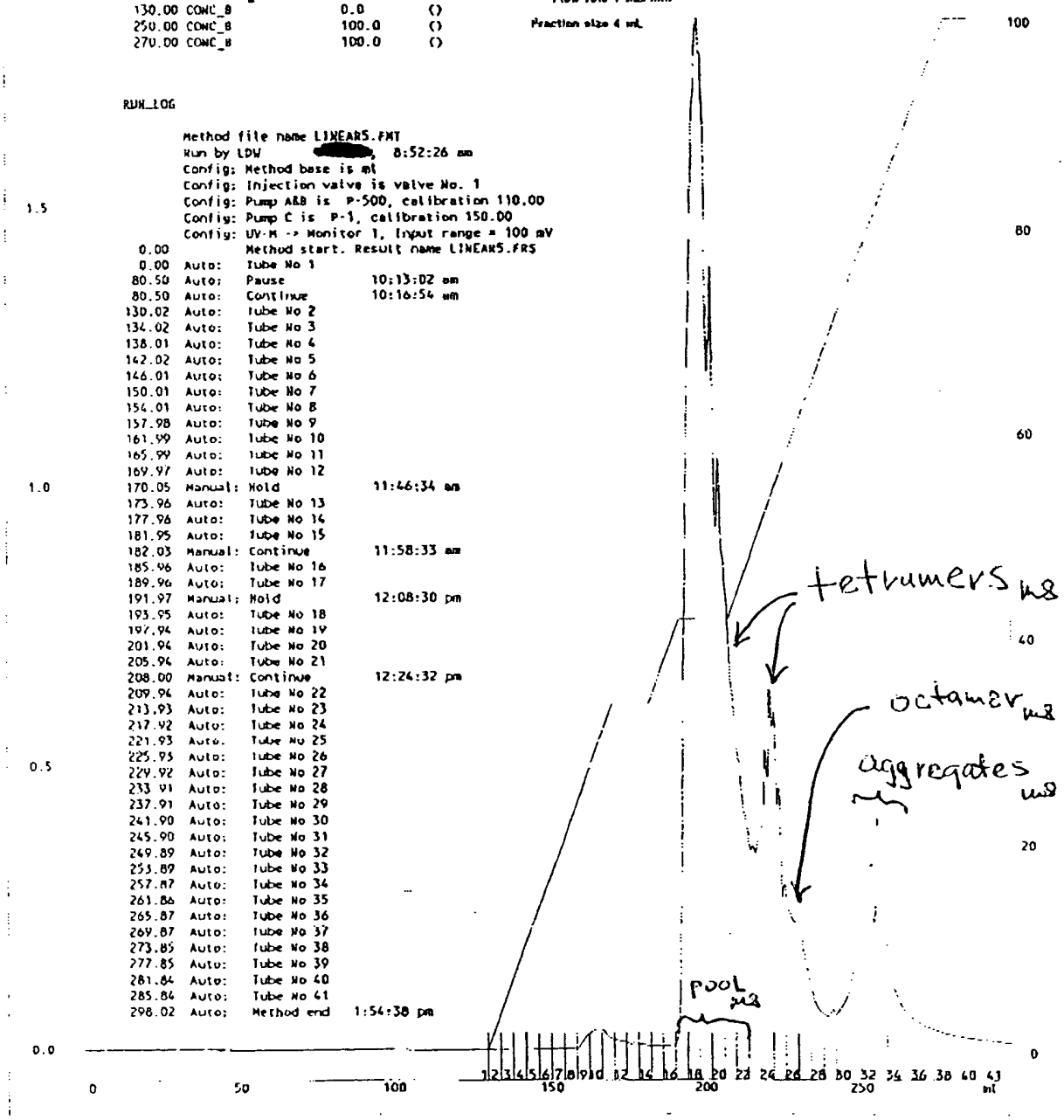

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EXHIBIT A PAGE 1 OF 4

Read and Understood By

Signed ____ Date ____ Signed [signature] Date ____

[redacted]. To flush Mono Q HR10/10 column in FPLC system with 0.6 M NaOH in 1 M NaCl, then water, then a blank run.
Buffer A = 0.1 M pH 10.3 sodium carbonate, Buffer B = 0.6 M NaCl / Buffer A.
[redacted]. Start loading 0.2 μ filtered BTG-Pig-KS-ΔN, lot AO-58-034 rec'd [redacted], from water-washed Nalgene filter unit, load 90 mL @ 1 mL/min directly then Pump A. About 8.5 mL remaining

gross filtered wt 154.6 g
net wet wt −62.4 g
92 g
1 mL rinse
−1.1 mL water/fills
94.3 g

8:34:50 start load
8:44:14 pause at end of 1st piston cycle.
8:52:04 start Method LINEAR5
10:12:38 pause method, stop load, start Buffer A
10:16:30 @ 80.5 mL continue
10:56:05 @ 120 mL
11:06:08 Start fraction collector, 4 min/fract = 4 mL/fract, at #2,
Hold gradient for three fractions at ~~41.6~~ 33.3 % B = 0.2 M NaCl
Hold gradient for 4 fractions at 41.6 % B = 0.25 M NaCl
13:34:14 reach 100 % B 250 mL
13:54:14 ramp off, end @ 270 mL

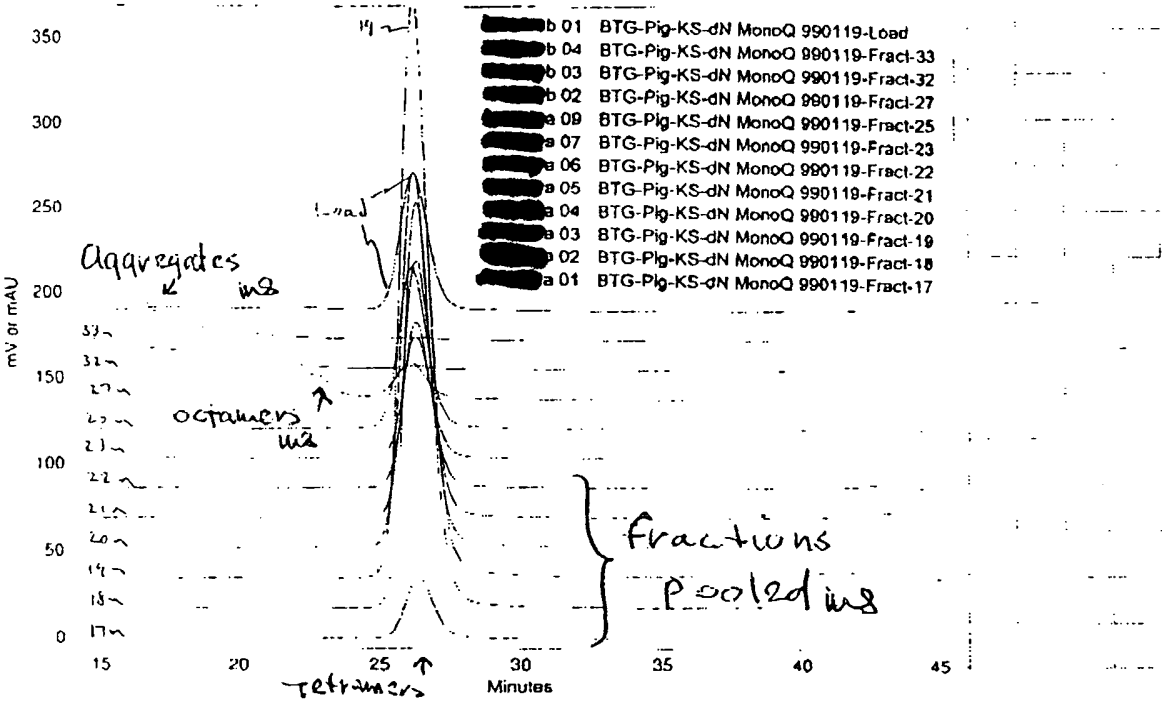

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EXHIBIT A PAGE 2 OF 4

Read and Understood By

Signed Date Signed Date

[redacted] Remove 180 µl returns of 19-53-Fract's 17-22 into HPLC vials w inserts. Also prepare dilutions as follows

17	1x	360µl return	Pool remaining 17-22 after return using fract 17 as wash of other to
18	1/8	50µl + 350µl ~~buffer~~ water now	Pool wt = 21.565 g
19	1/12	30µl + 330µl water	
20	1/8	50µl + 350µl water	Remove 2 mL Pool return
21	1/5	70µl + 280µl water	$(21.565 - 2) \times 10.6 \frac{mg}{mL} = 207$ mg target
22	1/3	100µl + 200µl water	

Weigh out 233 mg NPC-10K-PEG (Shearwater 4G-128-01) and add 0.932 mL 1mM HCl at 18:16. Momentary yellow color on solids before they are well suspended ∴ residual base in NPC-PEG? Wt of added HCl = 0.941 g. Add 1.099 g PEG-soln dropwise with stirring to the 19.5 ml Pool, ice bath, over 1-2 min. 1 mL PEG soln weighed 1.028 g. Calcd NPC PEG added = $233 \text{ mg} \times \frac{1.099}{0.941 + 233} = 218$ mg
Covered + refrigerated

[redacted] Remove µl sample of rxn for HPLC
Prepared 10K MidGee ultrafilter by rinsing with water, then 0.1 M NaOH
Rinsed with water, then 0.1M pH 10.3 buffer (left in lines, ie ~3 mL) and began ultrafiltering round 1 reaction at 16:10. By 16:23 ~~of~~ about 5 permeate had accumulated. Continue concentrating to minimum volu Stop at 17:37. Remove 3.266 g (~3.15 mL) retentate. Rinse apparat with 6 mL water, save separately.

[redacted] Begin stirring retentate in ice bath. Remove 110 µl sample + dilute with 140 µl (1/2) carbonate HPLC buffer at ~10:15. NPC-10K PEG weighed yesterday (344 mg initially, dropping to 337 mg with 1-2 mm static electricity?) + stored covered in freezer. Added 337 mg × 4 µl/1 × 902 = 1.22 mL 1mM HCl at 10:15. Again, yellow color on solid until well suspended. Refrigerate until add to rxn dropwise (~1 drop/sec) 10:54:23 – 11:04:10. Rinse tube with 0.12 mL mM HCl after reducing foam under partial vacuum. Add rinse to rxn at 11:07. Add 2 mL carbonate HPLC buffer to PEG-tube. UV_{400nm} @ 13:38 = $\frac{1.01}{18.4 \text{ AU/mm}} = 0.055 \text{ mm} \times 10 \frac{mg}{mL} =$
At 11:18:00 remove 20 µl rxn sample for UV monitoring at room temp. $t_{1/2}$ = 11.5 min (see LDW 012.14.KD). Drop 0.1 mm UV cell, breaking it. Dilute ~~10 µl UV soln~~ all recovered (< 20 µl) UV soln with 380 µl water (~1/20) for HPLC 990121.01. PEG1 submit calcd as 7.5) 20

Continued on Page 5

Read and Understood By

L D Williams — Signed
[redacted] — Date
Mark Shafer — Signed
Date

quench 2mL in 22 mL ~60 mM GlyGly (
Filter through 0.45μ filter. See [redacted] & 01 for HPLC.
~~Start ultrafiltration on 30K Millipore~~
Took wrong tube from refrigerator. ultrafiltering Round 1 permeate
No activity in retentate or permeate. Ultrafiltered 0.1 mg/mL BSA/50 mM
borax to try to chase off bound activity before discovering there was no
activity to chase off.
[redacted] Round 3: Removed 10μl sample of unquenched round 2 rxn
for dilution with 190μl water + HPLC.
Dissolved 10.4 mg NPC-10K-PEG in 0.36mL 1mM HCl and added
to the round 2 rxn at 17:23 with stirring in an ice bath. Rinsed in
PEG with 40μl 1mM HCl.

EXHIBIT A PAGE 4 OF 4

Continued on Page

Read and Understood By

Mark Saifer

Signed Date Signed Date